B5

42. The process as defined in claim 40 wherein the amount of said camptothecins-bearing trichomes falling away from said young vegetative tissues is reduced by [freezing] extracting freshly harvested matters said young vegetative tissues shortly after harvesting.

REMARKS

Claim Rejections ----35 U.S.C. 102

The objection of claims 1-3, 4-6, 27-29, 40-43, 46 and 47 under 35 U.S.C. 102 are respectfully traversed

Nature of Invention:

The present invention teaches methods to increase the yield of camptothecins in plants. Such increased yield is accomplished by increasing biomass growth ***and*** increasing the concentration of alkaloids through a precise combination of physical, biological, and ecological control of hormone production in the plant. The basis for the precise combination of steps called "Trichome Management" taught in the application revolves around the management and utilization of natural plant defense mechanisms. The specific Trichome Management techniques taught by the applicant are based upon applicant's hypotheses and discoveries which include:

(1) Alkaloids accumulate in glandular trichomes in leaf and stem surfaces;

(2) The alkaloids in trichomes play a major role in plant defense;

(3) Hormone levels determine the nature of alkaloidal defense (i.e., inter-inhibiting relationships between auxin and indol/qunoline alkaloids due to the same precursor tryptophan);

(4) Certain biological (i.e., genetic improvements), physical (i.e. T-pruning and pinching), and ecological (environmental stresses) applications effectively control plant hormones;

(5) The alkaloidal yield in young vegetative tissues (leaves and stems) is significantly increased by induced biomass growth and alkaloidal concentration increase through control of hormones; and

(6) Alkaloidal induction as plant defense consists of two steps: an emergent response to translocate alkaloids from old tissues to young ones, and a systematic response to increase alkaloidal contents in the whole plant.

These series of discoveries lie at the foundation of the claimed techniques. Although some of the cited reference may teach trichome or alkaloids are involved in the plant defense of *other* plants, they often teach away from the present invention and/or fail to teach what other steps are necessary to teach the very specific combination of steps claimed in the present invention.

In fact, simply knowing that trichome or alkaloids are involved in plant defense does not make the method to induce the alkaloids obvious. First, the alkaloids naturally accumulate in glandular trichomes only at certain stages of development but then transport the alkaloids to other tissues of plants. Second, only applications involving the promotion or inhibition of hormones will enhance alkaloids in plants. Third, even if successfully induced, the concentration of induced alkaloids change significantly with time, waning to levels before plant defense mechanisms are activated. Thus, timing the harvest of plant tissues is critical in increasing the yield of CPTs. The insight of the inventor in plant defense allowed him to make the above discoveries which are taught only by the present invention and not any combination of the cited references.

In sum, the present invention is novel and not taught or anticipated by any existing or any combination of existing teachings.

<u>State of the Prior Art:</u>

For decades, scientists have known that leaves contain CPT, although experiments showed contradictory results as to the level of CPT concentration in young leaves. Induction of CPT concentration and harvest of young leaves in *Camptotheca* has long been thought of

as a holy grail for inexpensive extraction of CPT. Many have tried, but all have failed or could not achieve the magnitude of result close to the present invention. Without the right motivation and understanding of the mechanism, those with ordinary skill in the art failed to provide a commercially feasible method for CPT production. For example, Vincent et al. method teaches a procedure yielding less than 6.0% of the CPT yield achieved through the present invention.

Vincent et al. (IDS reference #C-49) only teach the impact of pruning intervals on biomass growth: "by week 12, the total amount of CPT collected from trees harvested at 6-week intervals was 6.5 times greater than from trees harvested at 2-week intervals". These authors **do not teach that pruning can induce leaf biomass growth over non-pruning because** all trees in their experimental are clipped and **no control treatment** (i.e., no clipping and harvest at the end of 12 weeks) is used in their experiment. Their previous studies did not provide biomass data of unpruned trees to compare with the pruning in C-49 either. Thus, Vincent et al. will not lead those with ordinary skill to have a concept that pruning can increase leaf biomass in *Camptotheca*.

Vincent et al. clearly teach that pruning does NOT increases CPT concentration in leaves. First, Vincent et al. (C-49) teach that CPT concentration in the young leaves of **clipped trees** ranges from 0.45 mg/g to 3.49 mg/g (dry weight), which much lower than or equivalent to about 3.3 mg/g (dry weight) in **unpruned trees** in their previous studies (C-36) by the same group (the same seed source and similar age and size as in C-49). Further, Vincent et al. teach CPT concentration in the leaves stays constant after removal of apical bud. Obviously, their methods did not induce CPT concentrations at any time after pruning. This is an opposite teaching from that there are two peaks (steps) of induced CPT concentrations by pruning taught by the present invention. By reading all these articles, those with ordinary skill in the art will easily see that pruning has no effects on CPT concentration at all. **Obviously, Vincent et al. teach away from that CPT concentrations in *Camptotheca* can be increased by pruning.**

Because Vincent et al. failed to induce CPT concentration in leaves by their pruning method, they produce only 14.6 mg of CPT within 6 weeks (Fig.1 and the related text, Vincent et al.). The present invention teaches not just how to make more young biomass, but

also how to make each of those tissues produce more CPT. As the result, the present invention will produce **16 times MORE** CPT yield (247 mg) over the same time frame (see CPT yield at "3-week" in Fig. 24b of the present application).

Vincent et al. **have no motivation to teach the effects of auxin levels on CPT yield**. Vincent et al. do even not mention the word "auxin" in any of their publications on *Camptotheca*. Since all the experimental trees (C-49) were clipped, the auxin levels in all these trees are actually reduced, Vincent et al. give no motivation to study how CPT production is influenced by changing auxin levels. Although it is true that auxin levels may be controlled by pruning, without the theoretical foundation upon which the present invention is based, Vincent et al. would have no motivation to consider the level of auxin, especially since their teachings show no impact of pruning on CPT concentration at all. Without the theory upon which the present invention is based, no one with ordinary skill in the art, including Vincent et al. would be able to determine the specific timing of specific stresses necessary to achieve the results produced by the present invention.

Neither Vincent et al. nor Liu et al. provide any teaching or suggestion about trichomes or even mention the term. Actually, learning that pruning does not impact CPT concentration of leaves taught by Vincent et al., people with ordinary skill will easily conclude that **density of trichome** (where CPT accumulated) in the leaves will **not be affected by pruning**.

Li et al. (IDS reference #C-1) do not provide any statement on "trichome" because there was no knowledge of CPT accumulation in trichome during the time. The Office Action's statement " Li et al. which teach that camptothecin (CPT) is stored in the vacuoles of glandular trichomes" is part of the present invention and was stated in the unpublished internal report of the applicant in 2000 (Li et al., IDS reference #C-2). Without the internal report, there is neither motivation or prior art to show that the control of auxins would in any way affect CPT yield.

Because Vincent et al. teach away from increase CPT yield by control of auxin and none of the cited references provide any teaching or suggestion about trichome, **even the combination of the cited references will not lead to trichome techniques taught by the present invention**. The "trichome management" techniques are invented based on six

discoveries (see Nature of the Invention). Thus the present invention is patentable over the cited references.

There is no universal method to preserve plant materials for maximum extraction of targeted chemicals. Although each of the preservation methods has been commonly used in lab or industry, the selection of the method will depend on type of chemicals and plants. Therefore, several U.S. patents claim different drying method for different plant species. For example, it is claimed that drying treatment plant matters (between 20 and 70°C degrees) is the best method to preserve taxols in *Taxus* (Hala N. Elsohly et al. 1997, U.S. Pat 5,618,538). For tobacco, it is better to heat-dry plant matter to keep product quality (Joseph V. Fiore 1977, U.S. Pat 4,018,234; Yutaka Saito and Yuriko Anzai, 2000, U.S. Pat 6,109,272). For St John's wort (*Hpericum perforatum*) and echinacea (*Echinacea* spp.), vacuum microwave drying (<60° C) is claimed the best method to preserve the key active compounds in these plants (Timothy D. Durance et al. 2000, U.S. Pat 6,128,831). For CPT-containing plants, the present invention teaches either that freshly harvested matters or freeze-dried matters provide better CPT recovery than the above dry methods. As in many other plants, Vincent et al. (C-49) use commonly freeze-dried *Camptotheca* materials for their CPT analysis and do not teach that freeze-dry methods over any other. Liu et al. (C-34) teach traditional freeze-dried tissues are better than air-dry or oven-dry for CPT analysis. However, Liu et al. do not investigate freshly harvested matters and thus cannot compare the freeze-dry method with freshly harvested matters. **The applicant respectfully requests an amendment of the claim 42: change "freeze-dry method" to "freshly harvested matters."** Amended claim 42 is:

42. The process as defined in claim 40 wherein the amount of said camptothecins-bearing trichomes falling away from said young vegetative tissues is reduced by [freezing] extracting freshly harvested matters said young vegetative tissues shortly after harvesting.

The common ground methods of Vincent et al. and Liu et al. have limited effects on breaking cell walls of trichomes because the trichome walls are thicker than many other cells.

Thus, it is necessary to use the specialized methods in the present invention (Example 9) to target breaking the trichome walls for effective CPT extraction. The claimed methods (claims 46 and 47) are very useful to break the trichome walls and thus effective recovery of CPTs. Therefore, the applicant respectfully requests the present rejection be withdrawn.

Claim Rejections ----35 U.S.C. 103

The objection of claims 1-6, 10-12, 14-19, 21, 23-25, 27-32, 34, and 36-38 under 35 U.S.C. 103 are respectfully traversed

The present invention teaches a novel pruning (topping) technique to increase alkaloid yield via increasing both biomass growth and alkaloid concentrations in *Camptotheca* trees. Existing teachings for alkaloid induction by damage/pruning are limited to ***herbaceous plants*** and the results are contradictory. Topping as a pruning method is restricted to use in landscape shrubs and small trees and pruning experts always avoid applying topping to big trees since it may cause serve damage for tree species. Thus, skepticism on the effects of topping on alkaloid production in trees, particularly big trees, is obvious. Vincent et al. and Liu et al. tried to combine pruning with alkaloid production in *C. acuminata* but showed that either CPT content or biomass growth can **NOT** be increased by their pruning methods. The failed attempts teach people with ordinary skills away from the application of pruning techniques to induce alkaloid production in trees. Thus, even if the cited combination were proper, the present invention is patentably distinguished over the combination.

Teachings for alkaloid induction by damage/pruning in herbaceous plants are contradictory

It is not clear that if pruning induces alkaloids production in plants because the existing studies on the effects of pruning on alkaloid production in herbaceous plants are contradictory and misleading. Combination of McKey (IDS reference #C-117) and Medic (C-53) teaches that plants tend to respond to pruning (wounding or damage) by increasing alkaloid concentration in the wound area. The existing teachings on alkaloidal response by damage or pruning are basically restricted to herbaceous plants, particularly

annuals. For example, scientists have long known that damage to the flowering top of a tobacco (Nicotiana) plant increases its total alkaloid content (see Baldwin as C-73). However, the hypothesis on phytochemical induction by damage has been challenged by many authors (Fowler and Lawton as IDS reference #92). Fowler and Lawton (C-92) and Ralphs and Williams (C-129) show that the induced plant responses by damage are minimized even by using the same plant materials. Coleman and Jones (IDS reference #C-85) state that many studies on phytochemical induction contain large amounts of uncontrolled or unexplained variation within treatments, which has created controversial interpretation of results. For example, Fowler and Lawton (C-92) argue that there was insufficient evidence to support a defensive role for phytochemical induction because many experimental studies of induced resistance are poorly designed, are analyzed with inappropriate statistics, and have excessive amounts of variation within experimental treatments. The examples for the contradictory results are numerous. In lupine (Lupinus), some find that leaf damage increase quinolizidine alkaloids (Wink as C-145 and C-146, Bently et al. as C-75, Johnson et al. as C-102) while other researchers teach that no induced alkaliodal response at all by leaf damage (Ralphs and Williams as C-129). Baldwin (C-70) find that field grown wild tobacco plants have a fourfold increase in leaf alkaloids to respond to defoliation, but pot-bound plants are not inducible. General pruning attempts in *Camptotheca acuminata* by Vincent et al. (C-49) and Liu et al. (C-34) fail to teach that increase of either biomass or CPT yield by pruning. In a recent issue of **Science**, Agrawal (C-68) clearly teaches that the induced plant responses by clipping damage are minimized because of the absence of herbivore saliva. In another study, Mutikainen and Walls (C-119) even teach that three different nettle (Urtica) species (subspecies) have different responses to the stimulated herbivory treatments. **The skepticism on effects of damage or pruning on alkaloid production in *herbaceous plants* teaches people with ordinary skills away from the present invention.**

Further, teachings for herbaceous plants cannot apply to woody plants directly. Because of longer life span, more complex modularity and higher sectorality, woody plants have significantly different physiological responses to environmental stresses. Unlike herbaceous plants, woody plants survive above ground but usually stop growing to respond

unfavorable environments. Herbaceous and woody plants often experience different types of herbivory. For woody plants, it may take several years to develop a response to herbivory. Woody plants may also respond to hormones differently from herbaceous plants.

Common pruning techniques teach away from induction of alkaloid yield by topping of trees

The existing pruning techniques, particularly topping, are restricted to use in landscape shrubs and small trees. The techniques as in Medic (IDS reference #C-53), Avery (C-52), and Bedker et al. (C-51) or combination of the techniques may create a bushier plant habit and create more leaves thus increasing biomass growth in **small tree** species. The existing pruning methods teach that topping may increase biomass growth in shrubs or small trees and do not teach the impact of topping production of alkaloids or other chemicals.

The objective of the pruning is to produce strong, healthy, attractive plants (Bedker et al.). Because it may cause serve damage for **tree** species, topping (heading) is recognized as poor plant maintenance techniques. Even the topping techniques work well for small trees, existing teachings avoid applying topping to big trees like *Camptotheca acuminata* (up to 120 feet in height) as taught in the present invention. Bedker et al. clearly teach that "**topping and tipping are pruning practices that harm trees and should not be used**."

Because of **skepticism** on the effects of damage or pruning on alkaloid production is so obvious, motivation to combine alkaloid production with pruning is limited. Vincent et al. applied pruning in *C. acuminata* but teach that either CPT content or biomass growth can **NOT** be increased by their pruning methods. As a result, no commercial feasible pruning techniques have been developed to increase alkaloid production until the present invention.

Existing teachings on CPT contents in young leaves in *C. acuminata* are contradictory

Since the discovery of CPT by Wall et al. (IDS reference #C-26), studies on CPT variations among different *Camptotheca* tissues are numerous and often **contradictory** particularly on CPT content in leaf tissues (see **Table 1**). Even the teachings from the same group (Liu et al.) are different. Liu and Adams (C-31) teach that young and old tissues

within a growing season did not have significantly different CPT concentrations and leaves contain much lower CPT content than the branch, stem, or root. Liu et al. (C-34) then teach that CPT concentration in young leaf may contain 4-5 times higher than in the old leaf. **The results are contradictory** although all experiments are restricted to *C. acuminata* by the same authors. Without careful and further investigation, people with ordinary skills cannot make a decision. After the examination CPT contents of leaves in all different species and varieties of *Camptotheca* (all except *C. acuminata* are exclusively discovered, collected, or developed by the applicant), the applicant (Li et al. as C-2) teaches that the leaves of *Camptotheca* have the highest concentration of CPT of all the parts of the plant, which is the part of the present invention, not prior art.

Table 1. Different teachings on CPT concentration of young leaves

	Positive	Negative
Wall et al. 1966 (C-26)	Leaves have anti-tumor activity indicating contain CPT	
Perdue 1970 (C-44)		Leaf samples are inactive
Perdue et al. 1970 (C-45)		
Tien et al. 1977 (C-25)	Higher than roots and stems	
Cao et al. 1992 (C-41)	Young leaves contain enough CPT to kill goats and higher than fruits	
Lopez-Meyer et al. 1994 (C-36)	Young leaves > older leaves	
Liu et al. 1996 (CD-31)		Young and older tissues have no difference in CPT concentration. Leaves < branch, stem, or roots
Liu et al. 1997 (C-33)		More CPT in the bark, less in the wood and leaves
Liu et al. 1998 (C-34)	Young leaves > older leaves	

Failed attempts in *Camptotheca acuminata* by Vincent et a. and Liu et al.

As described in the present invention, **increase of CPT yield can be had either by increasing biomass growth or increasing CPT concentrations in plant tissues or both. Vincent et al. (C-49), Liu et al. (C-34), and Li and Adair (C-1) do not teach a commercially feasible pruning method to increase CPT yield.** Li and Adair only teach that coppicing can be applied in *Camptotheca* and do not teach that pruning can induce CPT yield. Although having motivation to combine Li and Adair with Medic's general pruning techniques, however, Vincent et al. teach that **neither** CPT concentration **nor** biomass

growth can be increased by pruning and CPT concentration stays constant over time after pruning. Thus, these failed attempts actually teach the people with ordinary skill away from applying pruning in *Camptotheca* to increase CPT yield. The present invention is distinguished itself from general pruning techniques in both methods and results.

In fact, the increase in biomass growth may cause either a decrease or increase of CPT concentration in plant tissues (see following examples) **or even no effect** (see Vincent et al.). For example, increased biomass growth causes decreased trichome density (=CPT concentration) under full sunlight (Fig. 20 of the present invention), and a similar pattern is found under irrigation treatment (Fig. 21 of the present invention). However, the invented T-pruning methods increase both biomass growth (Fig. 12 and Fig. 13 of the present invention) and CPT concentrations (Fig. 15 of the present invention), and a similar pattern is found in pinching method of the present invention (Figs. 16-18 of the present invention). Therefore, inducing both biomass growth and CPT concentrations to increase the total CPT yield is the unique advantage of the present invented T-pruning/pinching techniques over existing teachings and methods.

There are long-felt needs to increase CPT yield in *Camptotheca*. However, Vincent et al. failed to teach that pruning induces either biomass growth or CPT concentration over unpruned trees. Vincent et al. teach that CPT concentration in the leaves stayed constant after removal of apical bud and obviously their methods failed to induce CPT concentrations at any time after pruning. As a result, by using Vincent et al. method, within 6 weeks each plant produces only 14.6 mg, less than the **6%** of the present invention. Obviously, the commercial value of the Vincent et al. is very limited.

Liu et al. (C-35) fail to teach that coppiced shoots have higher CPT concentrations than not coppiced shoots under the same condition (e.g., the same tissue age). Liu et al. state that "our results show that shoots that develop as a result of coppicing have significantly higher leaf CPT concentrations than the original shoots. This confirms the previous findings that less mature *C. acuminata* tissues contain high CPT concentration." The second sentence clearly shows that the authors used relatively young "coppiced shoots" and relatively old "original shoots" in their comparison. It is now well accepted that CPT concentration in young tissues is much higher than old tissues. Clearly, as authors stated,

higher CPT concentration in coppiced shoots over original shoots in Liu et al. reference ((e.g., 0.0796 ± 0051 of coppiced shoots vs. 0.0650 ± 0.0047 of not coppiced shoots in August, Table 5 in Liu et al. C-35) **is mainly attributed by the age difference of shoot tissues rather than coppice treatments.**

Camptotheca acuminata can grow up to 120 feet in height; it is not possible to apply topping pruning techniques without clear motivation. Vincent et al. and Liu et al. have motivation to apply pruning in *C. acuminata* but failed to provide evidences of increase of CPT content by pruning. The experiment of Vincent et al. lacks using non-pruned trees as control and thus failed to teach either biomass growth or CPT yield can be induced by pruning, while Liu et al., as stated by themselves, the claimed slight increase of CPT concentration by coppicing is actually attributed by shoot age difference rather than coppice treatment. Further, Liu et al. teach that trees cannot produce high CPT yield after the juvenile stage by using common pruning techniques (16 times of decrease in CPT concentrations from 2 years old to 4 years old trees). Obviously, the Liu et al. teaching has very limited commercial value. Obviously, attempts to pruning *Camptotheca acuminata* to increase of either biomass or CPT yield by pruning failed. **Their data teach away from the pruning application to increase CPT yield.**

Combination of the cited references

Each of the cited references thus fails to teach or suggest the claimed invention. Common pruning techniques (Medic as C-53, Avery as C-52, and Bedker et al. as C-51) teach away from induction of alkaloid yield in **trees** by pruning. Existing teachings for alkaloid induction by damage or pruning in **herbaceous plants** are contradictory and misleading (positive results of Wink 1985 as C-145, Wink 1987 as C-146, Bently et al. 1987 as C-75, Johnson et al. 1988 as C-102, Baldwin 1991 as C-73 **VS.** negative results of Baldwin 1988 as C-69, Ralphs and Williams 1988 as C-129, Mutikainen and Walls 1995 as C-119, Vincent et al. 1997 as C-49, Liu et al. 1999 as C-35; Agrawal 1998 as C-68). Thus, motivation to combine cited references will teach away from the present invention. Because of such obvious skepticism on effects of damage or pruning on alkaloid production, to date no pruning techniques have been developed to induce alkaloid production until the present invention.

The attempts to combine **general pruning with alkaloid production in** *C. acuminata* by **Vincent et al. and Liu et al. failed to teach commercially feasible methods to successful induce CPT yield by pruning. Their failed attempts and critical mistakes in data collection and interpretation teach way people with ordinary skills from the application of pruning techniques to induce CPT production in *Camptotheca*.** Thus, even if the cited combination were proper, the present invention is patentably distinguished over the combination.

The present invention ("trichome management" techniques including T-pruning and pinching) is technically novel and commercially promising

The claimed techniques are invented based on the applicant's a series of new discoveries after elucidation of the existing puzzles. The present invention teaches how to increase CPT yield by inducing both biomass growth and CPT concentrations through novel "Trichome management" techniques including T-pruning/pinching. Clearly, to produce bushy habit by pruning is not a goal of the present invention because biomass growth usually may not increase alkaloidal concentrations.

The present invention teaches a novel method to increase CPT yield by accelerating growth of young leaves and stems and inducing CPT concentrations. Unique CPT analogs that are identified in non-treated plants can be induced because T-pruning techniques successfully control plant hormone (auxin) level in the plant and induction of plant defense. The present invention for T-pruning techniques has distinctive features from Vincent et al. (C-49) and Liu et al. (C-34) in pruning methods, harvest matters, and harvest time (**Table 2**). Both Vincent et al. or Liu et al. fail to teach a successful or commercially feasible pruning method to induce CPT yield. Vincent et al. do not teach that either biomass or CPT content can be increased by pruning. The present invention is distinguishable from Vincent et al. in all of motivation, pruning methods, and results. By using the invented T-pruning, the plant can produce 16 times higher CPT yield than Vincent et al. teaching. In addition to mistakes in experimental design and data interpretation, Liu et al. teach that "leaves should be harvested early in the growing season" because they found CPT concentration in leaves declines at 11% each month from April to October. According to Liu et al.'s teaching, it should replant the trees every two years to keep the high CPT yield because they teach 16

times lower in 4-yr-old trees than in 2-yr-old trees. But the present invention teaches a method to harvest plant matters in the whole growing season in a sustainable plantation system (8 years old plants still produce stable CPT yield). Additionally,

(1) Unlike the regular tree pruning applications, the T-pruning techniques significantly increase both biomass growth of young leaves and stems and induce trichome and CPTs production in *Camptotheca*.

(2) The compact form of *Camptotheca* trees is suitable for both manual and mechanical harvest methods.

(3) The compact form reduces the late frost damage, which allows *Camptotheca* plantation development in the southeastern United States.

Table 2. Differences of present invention in comparison with <u>Vincent et al.</u> and <u>Liu et al.</u>

	Vincent et al. (IDS Refer #C-49)	**Liu et al.** (IDS Refer #C-34, C-35)	**The Present Invention** (Li)
Pruning Objective	to increase CPT yield by increasing biomass growth	to increase CPT yield by increasing biomass growth	By controlling auxin levels (1) To enhance yield of CPTs via accelerating growth of young leaves and stems and inducing CPT cencentrations (2) To induce new CPT analogs
Problems Experimental Design	No un-prunned trees as control and all data were collected from different pruning intervals. Thus, no data show induction of either biomass or CPT yield by pruning over un-prunned trees	Use young coppiced shoots vs. older original shoots in the experiments. Thus, the difference in leaf CPT concentrations of coppiced and original shoots are basically attributed by tissue age rather than treatments	All these design problems are avoided. Data were collected from well designed experiments (from 1994 to 2000)
Biomass Growth	No data for pruning over control; Results: 6 weeks interval pruning better than 2 weeks interval	No data available	Yes. Significant.
CPT Concentrations	No increase by pruning (see C-36).	Data showed slight higher leaf CPT concentration	Significant increase T-pruning: +3-5 times

	"CPT concentration in the leaves stayed constant after removal of apical bud"	(14-18%) in coppiced shoots than original shoots. As authors stated, the difference is basically attributed by tissue age rather than treatments	Pinching: + 86% There are also two steps of CPT induction: emergence and systematic responses.
Leaf CPT Yield Variations (dry weight)	0.045% to 0.349% Excessive amounts of variations within treatments (one of the 6-week intervals group had a CPT concentration 10 times less than the others in the group)	0.050% to 0.131% Great variations due to no standard procedure applied	0.502% to 0.535% Stable yield because of the standard procedure used
New CPT Analogs	No induced	No induced	Yes. Due to systematic T-pruning, some novel CPT analogs that not existed in non T-pruning treated plants were identified
Pruning Method	Apical bud removal	No regular pruning Only ground coppicing	T-pruning including several prunings of all tips of branches, root pruning, and leaf pinching. within 60 cm of the ground
Pruning Times before Harvest	One	One	4 prunings to systematically induce CPT yield
1st Pruning Age	2 years old	2 years old	Starts at one year old
1st Pruning Time	No particular	No particular	After dormant period (immediately after the last frost in spring)
1st Pruning Height	No teaching. 150 cm in experiment	3 cm above the ground	within 60 cm of the ground
2nd Pruning Time	No	No	Within 3—5 months from the first pruning
3rd Pruning Time	No	No	2—3 months later
4th Pruning Time	No	No	2nd year after the last frost in spring
Leaf Pinching	No	No	Yes
Root Pruning	No	No	Yes
1st Harvest Age	No teaching	No teaching	A year later from the first pruning
Harvest Matters	Young leaves only	Young leaves only	intact clippings: young leaves attached to young stems (30 to 50 cm long)
Harvest Season	No teaching	Only early of growing season (1998)	Whole growing season
Tree Ages for harvest	No teaching	Only 2 years old (CPT concentration in 4 years old trees contain	Not limited. Both young and mature trees producing stable high CPT

		less than 1/16 of those 2 years trees) (1998)	yield
Total CPT yield per plant within 6 weeks	**14.5 mg**	**N/A**	**247 mg**

The objection of claims 7, 20, and 33 under 35 U.S.C. 103 are respectfully traversed

Cook (IDS reference #C-52) teaches root pruning methods only for "**shrubs**", which are obviously different from the big trees (up to 120 feet tall), the targets of the present invention. Further, Cook teaches how to keep a shrub in its proper habit because "root pruning is to reduce a plant's vigor or growth." Clearly, Cook's root pruning methods provide effective methods for maintain low status of landscape shrubs, but teaches away from the stimulation growth motivation of the present invention. The invented root pruning techniques not only stimulate the growth of the plants including roots but also to reduce the root rots problems identified in the *Camptotheca* plants.

Tobacco, the plant in all Baldwin studies (IDS references #C-69 to C-73), is an annual herbaceous plant species, which has different biological and ecological features from the woody trees involved in the claimed invention. In addition, more importantly, nicotine, a alkaloid in tobacco plants has a different biosynthetic pathway from CPTs involved in the present invention. Nicotine is a pyridine-piperidine alkaloid and derives from pyridine, while the CPTs are indole and qunoline alkaloids and derive from tryptophan. Baldwin (C-69), Dawson (C-69 page 48) and Mizusaki et al. (C-119) teach that nicotine first synthesis in roots and then transport to the leaves via the xylem. Thus, it is not difficult to realize that pruning roots to induce nicotine production in tobacco plants. In contrast, **CPTs accumulate in trichomes of leaves and shoots and have different biosynthetic pathway, and thus people with ordinary skill will have no motivation to use tobacco teaching or combine Cook or other pruning teachings and Baldwin with *Camptotheca*.** The claims 7, 20, and 33 of the present invention are clearly distinguished from the cited references or combination and are patentable.

The objection of claims 13 and 26 under 35 U.S.C. 103 are respectfully traversed

Baldwin (C-69) finds that tobacco plants respond more to mechanical damage than to true herbivory in terms of total leaf alkaloid contents. To understand the cause for difference

of mechanical damage and true herbivory, Baldwin designs four stimulated-herbivory treatments with **removal of 1-cm wide band of leaf tissues from the middle of the leaf excluding the midrib of the leaves on position 3 to 7 from the top of the plant**: 1) herringborne damage: gradual cut of leaf tissues between secondary veins; 2) serial damage: gradual cut perpendicular to the midrib without regard to vasculature; 3) vein damage: only the secondary veins from the midrib, and 4) control. He finds that treatment 2 resulted in significantly higher alkaloidal response than other. Baldwin (C-69, C-73) thus concludes that a plant's response to damage is determined by how one removes leaf area, not merely by the amount of leaf area removed. Baldwin (C-69, C-73) shows that leaf damages may induce alkaloidal production but he teaches effects of stimulated herbivory rather than pinching. According to Medic (C-53), "pinching" is a heading cut, used only on very soft tissues (young stem or leaf tips). However, Baldwin's leaf damages are restricted to a 1-cm wide band cut from the middle of leaf blade and thus do not involve leaf tips. Further, Baldwin's leaf damages are on the leaves on position 3 to 7 from the top and thus do not involve the stem tips. **Clearly, Baldwin (C-69) does not teach any pinching techniques**. Actually, in all his studies (C-69 to 73) Baldwin's interest in tobacco are **not on leaf or stem tips** because his investigations of alkaloidal response are focused on **mature leaves** (usually position 5 or occasionally position 8 from the top of plant), the harvest matters of the tobacco industry.

The invented pinching techniques focus on young leaf and stem tips where auxin highly concentrates. Tobaccos are small annual herbaceous plants, while *Camptotheca* spp. are big perennial trees. Unlike CPTs in *Camptotheca* which are accumulated in trichomes of leaves and stems and share a precursor trptophan with auxin as taught by the present invention, nicotine in tobacco is produced in roots and has a different biosynthetic pathway. Thus, Baldwin's leaf damage study of tobacco does not involve leaf or stem tips and his motivation is clearly to understand the effects of stimulated and true herbivory on alkaloidal response. **Balwdin has no motivation to teach people to induce alkaloidal response by controlling auxin level in plant**. In fact, Baldwin (C-72, C -73) clearly teaches that "although exogenously applied auxins clearly have a pronounced effect on alkaloid biosynthesis, a change in endogenous auxin concentration is probably not functioning as a negative cue that activates the response to damage."

The cited references (Vincent et al., Li, et al., Medic, McKey, Avery, Bedker et al., Purdue, Liu et al., Lopez-Myer et al., and Bryant et al.) do not provide any teachings on alokaloidal increase by pinching. Because nicotine is produced in roots rather than leaves and stems like CPTs, because nicotine derives from pyridine and thus does not involve trptophan (the precursor for both auxin and CPTs and thus there is inter-inhibiting relations between auxin and CPTs), because mature leaves are targeted harvest matters in tobacco plants (vs. young leaves and stems in *Camptotheca*), **Baldwin (C-69 to 73) does not teach pinching techniques involving leaf or stem tips, and he further has no motivation to relate leaf damage to auxin control. Because the obvious differences of tobacco and *Camptotheca* plants and their alkaloids, people with ordinary skill will have no motivation to use teachings in tobacco to *Camptotheca*. Even if people have motivation to combine the cited references, Baldwin's negative result of endogenous auxin on alkaloidal response teaches away from the pinching techniques involving auxin of the present invention.** Therefore, the applicant respectfully requests the rejection of claims 13 and 26 be withdrawn.

The objection of claims 22 and 35 under 35 U.S.C. 103 are respectfully traversed

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Baldwin (C-69) finds that tobacco plants respond more to mechanical damage than to true herbivory in terms of total leaf alkaloid contents. To understand the cause for difference of mechanical damage and true herbivory, Baldwin designs four stimulated-herbivory treatments with **removal of 1-cm wide band of leaf tissues from the middle of the leaf excluding the midrib of the leaves on position 3 to 7 from the top of the plant**: 1) herringborne damage: gradual cut of leaf tissues between secondary veins; 2) serial damage: gradual cut perpendicular to the midrib without regard to vasculature; 3) vein damage: only the secondary veins from the midrib, and 4) control. He finds that treatment 2 resulted in significantly higher alkaloidal response than other. Baldwin (C-69, C-73) thus concludes that a plant's response to damage is determined by how one removes leaf area, not merely by the amount of leaf area removed. Baldwin (C-69, C-73) shows that leaf damages may induce alkaloidal production but he teaches effects of stimulated herbivory rather than pinching. According to Medic (C-53), "pinching" is a heading cut, used only on very soft tissues (young stem or leaf tips). However, Baldwin's leaf damages are restricted to a 1-cm wide band cut from the middle of leaf blade and thus do not involve leaf tips. Further, Baldwin's leaf damages are on the leaves on position 3 to 7 from the top and thus do not involve the stem tips. **Clearly, Baldwin (C-69) does not teach any pinching techniques**. Actually, in all his studies (C-69 to 73) Baldwin's interest in tobacco are **not on leaf or stem tips** because his investigations of alkaloidal response are focused on **mature leaves** (usually position 5 or occasionally position 8 from the top of plant), the harvest matters of the tobacco industry.

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The objection of claims 43 and 44 under 35 U.S.C. 103 are respectfully traversed

Without knowledge of trichomes as CPT acumination sites in *Camptotheca* Van Hengel et al. only teach a sonification method for regular stem cells rather than for trichomes. The present invention shows that trichome cell walls are much thicker than regular cell walls so the method to treat trichomes shall be different from the Van Hengel et al. Thus the present invention is distinguishable from Van Hengel et al.

The objection of claims 43 and 45 under 35 U.S.C. 103 are respectfully traversed

Gershenzon et al. (IDS reference #C-94) teach a method of extracting the contents of glandular trichomes of peppermint with a cell homogenizer. Vincent et al. (C-49) do not teach any trichomes as CPT accumulation site, which is part of the present invention. Without knowing trichome as CPT accumulation sites in *Camptotheca*, people with ordinary skill will have no motivation to combine Gershenzon et al. with Vincent et al. or any teachings on *Camptotheca*. The present invention teaches a very useful method (see Example 9, Fig. 28a and Fig. 28b) to break the trichome walls and thus effective recovery of CPTs. Therefore, the claims 43 and 45 of the present invention are patentable without motivation of combination of the cited references.

CLOSING

As the instant invention has now been sufficiently distinguished from the references, the claims are now in a condition for allowance. Such action upon reconsideration of the Examiner is earnestly solicited. A clean copy of the pending specifications is enclosed as Attachment "A". A clean copy of the pending claims is enclosed as Attachment "B".

Should the Examiner feel that the prosecution of the above-identified application may be materially advanced by a telephone call, he is hereby requested to contact the undersigned.

Should there be any additional fees required, please charge such additional fees to Deposit Account 10-0447.

Respectfully submitted,

JENKENS & GILCHRIST

Alan R. Thiele, Regis. No. 30,694
112 East Pecan Street, Suite 900
San Antonio, Texas 78205-1533
(210) 246-5657 (Phone)
(210) 246-5999 (Fax)

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